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A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY
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EXPERIMENTAL STUDIES ON THE TOXICITY OF PROCAINE[1]

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The toxic effects of the local anesthetics are due mainly to three actions, the most important being a depression or paralysis of respiration. This is the usual cause of death in procaine poisoning. The second effect is a depression of the circulation resulting in a fall in blood pressure. The third effect is on the central nervous system, possibly the cerebral cortex, and is manifested by the production of convulsions which may be followed by a state of depression.

Procaine poisoning usually results in respiratory paralysis followed by delayed cardiac failure which many workers think is due to asphyxia rather than to direct depression of the heart, since the animals can be revived in most cases by prompt administration of artificial respiration, as shown by Tainter and Throndson (1). Potassium cyanide, in doses which markedly stimulate respiration has been demonstrated by Knoefel, Herwick, and Loevenhart (2) to have very little effect in the respiratory depression after procaine administration. They concluded that respiratory stimulants were of no value in the therapy of intoxication from local anesthetics. However, metrazol has been successfully used by Zipfe and Hoppe (3) to counteract procaine in white mice and cats when the 2 drugs were given simultaneously.

This paper deals with the acute toxicity of procaine in the dog and possible antidotal agents, together with observations on the development of tolerance. In all the experiments to be described, the term "procaine" refers to procaine hydrochloride.

[1] From a thesis submitted in partial fulfillment of the requirement for the Ph.D. degree, University of Chicago. (Received for publication April 7, 1941.)

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PROCEDURE AND RESULTS

Acute Toxicity. Lethal doses were determined on dogs anesthetized with 2 cc./kgm. of paraldehyde orally. The vagi were sectioned, blood pressure recorded in mm. Hg. and respiratory movements recorded by means of a pneumograph and tambour. Repeated doses of increasing size of 2 per cent procaine were injected intravenously at a rapid rate until the lethal dose was reached. At least 20 minutes were allowed to elapse between injections to minimize cumulative effects, as Hatcher and Eggleston (4) have pointed out that a fatal dose of procaine is destroyed in 20 minutes by the liver. In 20 dogs, the mean lethal dose was 9.7 ± 3.8 mgm./kgm. ($s.d._m = \pm 0.85$). There was an abrupt fall in blood pressure and depression of respiratory rate and amplitude as has been observed previously. In most cases, the circulation was maintained for a few minutes after respiratory movements had ceased. This was in accord with Isenberger's (5) results after intravenous injections of procaine in the dog. On the other hand, Knoefel, Herwick, and Loevenhart (2) reported that procaine intravenously caused a lowering of blood pressure with absence of vagal stimulation, and that the failure of the heart was the primary cause of death from intravenously administered procaine in dogs under amytal anesthesia.

Lethal dose determinations were also made upon unanesthetized dogs, continuously injected with 2 per cent procaine until death. The injection was kept as constant as possible at 5 cc./min. The symptoms seen were those commonly described by many workers after parenterally administered procaine (6), (7), (8), (9); excitation, increased extensor tone that usually began at the hind legs and traveled anteriorly, running movements, convulsions, and respiratory failure. The apparent lack of balance in these dogs seemed to be merely an effect of increased extensor tonus. The convulsions observed were of a clonic type and were preceded and followed by running movements, the duration of the attack depending upon the dose. With our methods, the mean dosage to produce excitation was 26 ± 6 mgm./kgm., running movements 27 ± 8 mgm./kgm., and convulsions 41.5 ± 9 mgm./kgm. The mean lethal dose was found to be 62.4 ± 14.6 mgm./kgm. ($s.d._m = \pm 2.7$) from injections into 30 dogs. This fatal dose may be somewhat higher than is ordinarily obtained because

of the method of administration, but is, nevertheless, reliable for the comparisons of the remainder of the study where the same technique was used. If any overshooting occurred, it probably was not of great magnitude as indicated by the rapid deaths of each of 9 dogs after injections of doses only slightly larger than those which the dogs were known to tolerate.

Repeated Administration. Suggestion of a tolerance to procaine was observed in the reactions of one unanesthetized dog who was given graded increasing doses of 2 per cent procaine at a rate of 5 cc./min.

TABLE I

Effect of repeated intravenous injections of procaine on the appearance of running movements

DAY	DOSE GIVEN (MGM./KGM.)	MGM./KGM. AT WHICH RUNNING MOVEMENTS APPEARED
23rd	45	38
25	50	39.1
27	50	41.4
29	55	43
32	60	no record
34	65	46
45	70	35
47	75	40
49	75	42
52	80	51
54	85	49
56	90	55
59	95	no record
61	108	57
98	92 (injection until death)	28

about every 2 days. The starting dose was 4 mgm./kgm. and each dose was increased by 5 mgm./kgm. It was found that the dose necessary to produce running movements became larger each succeeding time provided the 2–3 day interval was used, but that when 11 days elapsed between injections, the running movement dose dropped to below the original level. After a total of 21 injections, this dog had running movements at 57 mgm./kgm. and survived 108 mgm./kgm. of procaine (Table I). To study this phenomenon further, 9 additional dogs were subjected to the same procedure. The mean lethal dose for these animals was 61 mgm./kgm. which is well

within the range of the dogs which had had but a single injection of procaine.

The excitatory state in these dogs appeared when 10–15 mgm./kgm. of procaine had been injected as compared with 26 mgm./kgm. for the animals given but a single injection. But, succeeding larger doses induced an excitation which appeared at higher and higher levels, in some cases after a preliminary drop, with an apparent maximum nearly two to three times that of the initial excitatory dose. In a few cases where pupil size was measured, it was seen that during the course of a large injection, the pupils dilated until after the excitatory stage. Just before the onset of convulsions, the pupils began to constrict and remained in the constricted state throughout the period of convulsions. Pupillary dilatation after parenterally administered procaine has been described by MacGregor (10) but no description of the constriction that was observed when larger doses were injected could be found.

Thus it was found impossible to induce tolerance to the lethal effects of intravenous procaine in 9 dogs by the same procedure which had induced an apparent tolerance in one dog. However, with the repeated injection of the drug there occurred an increase in the dose necessary to produce excitation, which might be interpreted as one indication of acquired tolerance. This phenomenon was unexpected in view of the reactions of animals to repeated administration of many other convulsant drugs.

On the other hand, rats which were subjected to a similar procedure showed increasing susceptibility to procaine rather than any tolerance. A series of 33 rats were given graded, increasing, intraperitoneal doses of 2 per cent procaine solution every 2 days, starting with 40 mgm./kgm. and increasing each dose by 20 mgm./kgm. The convulsant dose was 160 mgm./kgm. and all the rats were killed by 200 mgm./kgm. Essentially the same results were found in 15 rats subjected to this procedure daily instead of every 2 days. That these data indicate a much greater susceptibility in the treated animals is shown by the fact that 10 previously uninjected rats under the same environmental conditions showed only a 20 per cent mortality when injected with 200 mgm./kgm., and 30 others gave an 80 per cent mortality from 300 mgm./kgm., the value reported by Rose, Coles, and Thompson (11) to be the median lethal dose.

These experiments on rats were performed in the hot summer months. Sievers and McIntyre (12) have shown that the toxicity of procaine for white mice increases with the temperature, and caution about administration of procaine during the summer. Mice are known to have poor temperature regulation, but to exclude any possibility of the temperature having been a factor in the rats, the entire experiment was repeated in the winter months. That the temperature affects the toxicity in rats as well as mice was shown by the convulsant dose in the rats in the winter group being 200 mgm./kgm. and all being killed by 280 mgm./kgm. Nevertheless, there was still evidence of increased susceptibility from repeated injections since 10 normal rats under the same environmental conditions showed only a 40 per cent mortality at 280 mgm./kgm. In terms of median lethal doses, the treated rats had an M.L.D. of 220 mgm./kgm. as compared to 285 mgm./kgm. for the controls. The above experiments demonstrate that increased susceptibility developed with a progressive increase in the dose administered.

Whether the same phenomenon would result from repeated injections of a fixed dose remained to be determined. Accordingly, daily intraperitoneal injections of 100 mgm./kgm. as 2 per cent procaine were given to 166 rats for various periods of time up to 5 weeks. This dose, which produced no symptoms at first, soon resulted in the appearance of spastic hind legs and running movements. By the end of 5 days of daily injections, the majority of rats showed convulsions, which were present every day. At the end of the injection period, these rats were given 300 mgm./kgm. of procaine intraperitoneally, the mortality being 85 per cent for both these rats and for 30 normal rats from the same colony. It would appear, therefore, that repeated administration of fixed doses leads to a greater susceptibility with respect to convulsions but not to mortality. In this respect, procaine is not unique since other convulsant drugs are known to lead to a greater susceptibility from repeated administration of equal doses.

Circulatory Effects. The fall in blood pressure after intravenous procaine injections and subsequent circulatory failure was reported by Knoefel, Herwick, and Loevenhart (2) to be the primary cause of death in dogs under amytal anesthesia. The mechanism of this fall in blood pressure is uncertain. Eggleston and Hatcher (8) are of the

opinion that this fall in blood pressure is due to a weakening of the heart and not any immediate vasodilatation. By using perfused frog legs and measuring blood outflow from the abdominal veins, Swanson (13) reported that while cocaine produced a constriction in dilutions of 1:1000 and 1:2500 and a dilatation in dilutions of 1:5000, procaine in the same concentrations produced neither a constriction nor a dilatation. To determine the effects of procaine on the peripheral vascular bed, the blood flow through the hind limbs of dogs under pentobarbital anesthesia was measured by a modification of the technique described by Lawson and Holt (14). Instead of the differential manometer used by these workers, small bore mercury manometers were connected to the femoral artery above a constriction and to a small branch below it. With this procedure, injections of vasoconstrictor or vasodilator substances made into the artery above the constriction will cause a change in blood flow which can be measured by changes in the differential of pressure. At the end of the experiments, whenever possible, the constrictions were calibrated by measuring, directly, the flow below the constriction necessary to produce manometer deflections in the ranges observed during the experiments.

In each of 8 dogs, procaine was observed to be a peripheral vasodilator. 0.1–50 mgm. of procaine in 2 per cent solution, injected intra-arterially, caused an increased blood flow through the dog's limb. (*fig. 1*) The response to injections became more variable after a large number of injections had been given so that no quantitative comparisons could be made. Although procaine may cause weakening of the heart as Eggleston and Hatcher (8) believe, the peripheral vasodilatation after procaine administration is a definite factor in the immediate fall in blood pressure.

The circulatory effects of procaine were not great enough to cause death in these as well as the other animals, the circulation being maintained after respiratory movements had ceased.

Respiratory Stimulants as Antidotes. Since the depression of respiration was found to be the primary effect of procaine administration, several respiratory stimulants were tested for their antidotal power. Alpha lobeline, caffeine, benzedrine, and metrazol were administered to both normal and anesthetized dogs simultaneously with the procaine or at the time of respiratory depression or stoppage. The

efficacy of artificial respiration in reviving dogs whose respiration had been stopped by procaine was also tested.

Twelve dogs under paraldehyde intravenously were rapidly injected with 10 mgm./kgm. of procaine in 2 per cent solution to which 100–300 mgm. of metrazol in 10 per cent solution had been added. Seven dogs survived the injection of this mixture. After 20 minutes to an hour, the 7 survivors were injected with the same dose of procaine

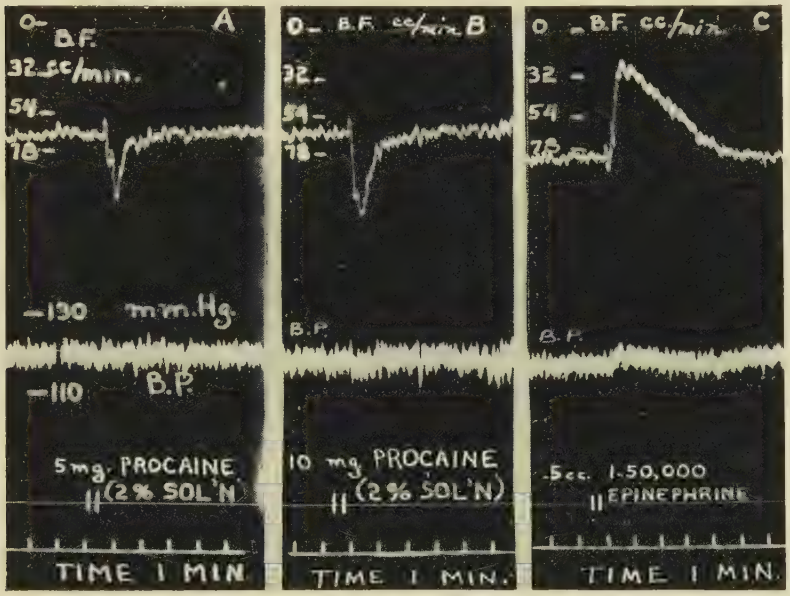

FIG. 1. Effect of intra-arterial injections of procaine and epinephrine on bloodflow through the dog's hind limb. B.F. = blood flow. B.P. = blood pressure.

alone, and 5 died. The 2 remaining dogs were given 15 mgm./kgm. procaine plus metrazol which injections proved to be fatal.

Since metrazol appeared to have some protective action in these dogs the drug was tried on normal unanesthetized dogs which were given 80 mgm./kgm. procaine (as 2 per cent solution) intravenously at a rate of 5 cc./min. To this solution, 100–300 mgm. of metrazol in 10 per cent solution were added. The mean lethal dose, 59 mgm./kgm., fell into the range already determined for procaine alone. By adding the metrazol to the procaine, the mean convulsant dose was

lowered to 19 mgm./kgm. Both metrazol and procaine are convulsant drugs and together they have additive actions. Although no lowering of the convulsant level was observed when only 0.2 mgm./kgm. of metrazol were added to the procaine solutions, there was no evidence of significant antidotal action in each of 6 dogs.

Although one-half of the anesthetized animals responded favorably to metrazol, it was concluded that metrazol could not have any clinically significant protective action against procaine, since it had no effect in counteracting the toxic effects in unanesthetized dogs, and its convulsant property is a definite disadvantage.

No success in reviving dogs was obtained from injections of metrazol caffeine, alpha lobeline, and benzedrine in doses which normally stimulate respiration in the dog, when the injections were made at the time of respiratory depression after lethal doses of intravenously administered procaine. Administration of artificial respiration, immediately after respiratory movements had ceased, was usually successful in reviving dogs. In some cases, the addition of epinephrine intravenously was necessary.

These experiments demonstrate that the respiratory stimulants tested had little value as antidotes against procaine intoxication. Although this work was not undertaken to determine the site of respiratory paralysis in procaine poisoning, the recent work of Bonnycastle (15) who showed that the phrenic nerve roots in the spinal dural sac were paralyzed by concentrations of procaine which the respiratory center in the medulla withstands, also indicates the futility of using central respiratory stimulants as therapeutic agents in procaine intoxication.

SUMMARY AND CONCLUSIONS

1. The mean lethal dose of 2 per cent procaine hydrochloride solution injected intravenously at a rapid rate in 20 dogs anesthetized with paraldehyde, was found to be 9.7 $\pm$ 3.8 mgm./kgm. ($s.d._m = \pm.85$).

2. In 30 normal unanesthetized dogs, the mean lethal dose of 2 per cent procaine injected intravenously at a rate of 5 cc./min., was found to be 62.4 $\pm$ 14.6 mgm./kgm. ($s.d._m = \pm 2.7$).

3. No apparent tolerance to the lethal effects of procaine could be induced in dogs by the repeated intravenous injections of graded

increasing doses of the drug. An indication of a tolerance with respect to the symptoms was obtained.

4. Susceptibility to procaine was increased in rats by repeated intraperitoneal injections of graded increasing doses of the drug. As in mice, procaine toxicity increased with a rise in environmental temperature.

5. Like some of the other convulsant drugs, procaine susceptibility was increased in rats by repeated injections of equal doses of the drug.

6. In dogs under pentobarbital anesthesia, intra-arterial injections of procaine in doses of 0.1–50 mgm. acted as a peripheral vasodilator as measured by blood flow through the hind limb. This vasodilatation is probably a factor in the immediate fall in blood pressure after intravenously administered procaine.

7. In both unanesthetized and anesthetized dogs, moderate doses of metrazol injected intravenously and simultaneously with lethal doses of procaine, were found to have little clinically valuable antidotal action. In dogs anesthetized with paraldehyde, metrazol, caffeine, alpha lobeline, and benzedrine had no effect in restoring respiration after lethal doses of procaine. Prompt administration of artificial respiration immediately after cessation of respiratory movements was usually successful.

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